Atty. Dkt. No. 078556-0143

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Petr Dobrovolny
Title:	METHOD OF MANUFACTURING OF 7-ETHYL-10-HYDROXYCAMPTOTHECIN
Appl. No.:	10/582,650
International Filing Date:	12/14/2004
371(c) Date:	6/13/06
Examiner:	Charanjit Aulakh
Art Unit:	1625
Confirmation Number:	1526

DECLARATION OF PETR DOBROVOLNY UNDER 37 C.F.R. §1.131

Dear Examiner Aulakh:

I, PETR DOBROVOLNY state and declare that:

1. I am the inventor of the invention recited in claims 23-37 of the patent application identified above. I made my invention while working in the Czech Republic.

2. Exhibit A is a copy of pages 72, 84, 88 and 89 from my research notebook, No. 768. My notebook entries on these pages are written in Czech. Exhibit B is an English translation of the four notebook pages. I have reviewed the translation and find that it accurately represents the content of my notebook entries. The pages describe the preparation of 7-ethyl-1,2,6,7-tetrahydrocamptothecin and 7-ethyl-10-hydroxycamptothecin according to methods that

are now the subject of claims 23-37. Exhibit C (C1-C4) shows HPLC Chromatograms for the compounds I synthesized, which are described by the notebook pages of Exhibit A.

3. Page 88 of my notebook describes the procedure I used to hydrogenate 7-ethyl-camptothecin to provide 7-ethyl-1,2,6,7-tetrahydrocamptothecin. In the procedure, the equipment and amounts of reactant used were the same as that listed on pages 72 and 84, respectively, of the same notebook (also shown in Exhibit A). I used 5% platinum on carbon (0.022 equivalents of Pt with respect to starting material) in acetic acid (989 equivalents) and in the presence of a small amount of dimethylsulfoxide (0.28 equivalents). The hydrogenation was carried out with stirring (900 rpm) under hydrogen at a pressure of 5 atmospheres, at 65 °C for 43.5 hours. After the reaction was terminated, the catalyst was filtered off and washed with 10 ml acetic acid (141 equivalents), and the combined acetic acid solution used in the oxidation described on page 89 of my notebook. I submitted a sample of the hydrogenation product for analysis by high pressure liquid chromatography (HPLC). The resulting chromatogram of the hydrogenation product is shown in Exhibit C1. The product was compared to a reference standard and identified as 7-ethyl-1,2,6,7-tetrahydrocamptothecin, present as three diastereomers.

4. Page 89 of my notebook describes the procedure I used to oxidize 7-ethyl-1,2,6,7-tetrahydrocamptothecin to provide 7-ethyl-10-hydroxycamptothecin. The acetic acid solution (80 ml AcOH, 1130 equivalents) of 7-ethyl-1,2,6,7-tetrahydrocamptothecin from the hydrogenation described on page 88 was combined with 22 mL of water (983 equivalents) and iodobenzene diaceate (1.89 equivalents). The mixture was stirred for 15 minutes, concentrated, the residue mixed with acetonitrile and filtered. I submitted three samples from this experiment for HPLC analysis: oxidation reaction solution, the crystalline product and mother liquor. The resulting chromatograms of the samples are shown in Exhibits C2, C3, and C4, respectively. The crystalline product was identified as 7-ethyl-10-hydroxycamptothecin by comparison to a reference sample.

5. Although the dates of my notebook pages are redacted, the notebook pages are dated prior to May 12, 2003 and after the year 1995. In addition, the HPLC analysis shown in

Exhibit C were run prior to May 12, 2003 and after the year 1995. Collectively, Exhibits A, B and C show that I reduced to practice and invented the subject matter of claims 23-37 prior to May 12, 2003.

6. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the above-referenced application or any patent issuing thereon. All statements made of declarant's own knowledge are true and all statements made on information and belief are believed to be true.

Petr Dobrovolny

Petr Dobrovolny

11.4.2008

Date

EXHIBIT A

72

HYDROGENACE 7-ET-CPT

[redacted]

Nářadí: 0,5 g 7-ET-CPT (768-65-1)
0,32 g 5% Pt/C (Š.05002 PI)
0,025 ml DMSO (S. 432316/1)
70 ml CH_3COOH (Š. 20810)

Aparatura: Čtyřkrkový autokláv, N_2, H_2, kádinka, váženka, odměrný válec, mikropipeta, odsávačka s baňkou,

Postup: Do kádinky jsem navážila 7-ET-CPT, do váženky Pt/C a pak přidala do kádinky. Zalila čistou CH_3COOH a dala do N_2 z homogenizovat. Pak jsem přidala DMSO, Promíchala a převedla do autoklávu, zbytky splachla zbylou CH_3COOH.
Autokláv se zkompletoval, pustila otáčky 500 ot/min, propláchla N_2 (3x), H_2 (3x) a pak se pustil H_2. Otáčky míchání se zvýšily na 1000 ot.

Hydrogenace začala v 10^{00} hod při 50°C, v 11^{40} hod 62°C, v 10^{52} hod 64,2°C.

tlak 5 atm. H_2, míchání 1000 ot./min, t = 63-65°C

Teplota díky puštěné chl. vodě ubývá o 2°C méně, takže se ve 12^{40} h nastavila + na 67°C. Ve 12^{55} h t = 64,9°C.

V 16 h zastavena hydrogenace. Filtrována a promyta. Nyní v lednici.

[redacted]

V lednici ztuhlé, teplou vodou vypuštěno.
768-72-1 Odebrán vzorek na HPLC (20 µl):
N-mi 80 ml ~~Et~~ 7-ET-THCPT

HYDROGENACE 7-Et-CPT

[redacted]

Násada: 0,5 g 7-Et-CPT (768-65-1)
0,32 g 5% Pt/C (š. 05002 PI)
0,025 ml DMSO (š. 432316/1)
70 ml CH_3COOH (š. 20 810)

Aparatura: viz str. 72

Postup: Do nádobky se navážil 7-Et-CPT, do vážky Pt/C a pak se přidal k 7-Et-CPT. Zalilo se 1/2 celkového množství CH_3COOH a zhomogenizovalo v UZ.* Přelilo do autoklávu a spláchlo zbytkem octové kys. Aparatura se uzavřela.
* přidal se DMSO.
Propláchlo se N_2 a H_2.
Hydrogenace spuštěna v 8[47] h.
Míchání 900 ot/min, tlak 5 atm. H_2
Vyhřáto na 65 °C v 9[50] hod.

V 9[10] hod = 64,2 °C

[redacted]

Konec hydrogenace v 9[50] hod. Hydrogenovalo 24 hod. (při 65 °C)

v 10[15] h Filtrace a promytí CH_3COOH (5 ml). Něco prošlo, muselo se znovu filtrovat a propláchnout (spotřeba celkem asi 40 ml CH_3COOH)
768-84-1) N = 124 ml 7-Et-THCPT. Vzorek HPLC: 64,94% 7-Et-THCPT; 7,9797 Et
(Světle oranžový až tmavě žlutý ☺)

PATŘÍ K STR. 85

Postup: V 11[40] h Nalito do 3hrdlé baňky (250 ml), přidalo se 35 ml demivody a dávkuje se IBDA: (Míchá se při 600 ot/min)

1. 0,613 g	v 11[42] h	tmavě oranžové	po 1/2 hod
2. 0,15 g	ve 12[12] h	—"—	po 50 min
3. 0,13 g	ve 12[55] h	—"— až hnědé	po 30 min
4. 0,04 g	ve 13[27] h	tmavě červenohnědé	

[redacted]

HYDROGENACE 7-E7-CPT

Násada: viz str. 84

Aparatura: viz str. 72

Postup: Do kádinky jsem navážila 7 E7-CPT a přidala Pt/C. Poté čistý CH_3COOH a zhomogenizovala na UZ. Přidání Pt/C po promíchání vlila do autoklávu. Vypláchla zbytkem CH_3COOH. Autokláv se uzavřel, pustilo se míchání, vyhřívání a propláchl H_2 a N_2.

Hydrogenace začala v 13^{25} hod.

Vyhřáto na 65°C ve 14^{00} hod.

Míchání = 700 ot./min, tlak = 5 atm. H_2, teplota = 65°C.

[redacted]

Konec hydrogenace v 9^{30} hod. Hydrogenace při 65°C **43,5 hod.**

Ochlazeno na 25°C. Filtrace a promytí CH_3COOH

768-88-1 √ V = 80 ml 7-Et-THCPT. HPLC (30 µl): 65,80% 7-Et-THCPT, 8,23% 7-Et-CPT

Světle žlutý, při filtraci přechází na hnědé zbarvení.

[redacted] Mohelská! Dobra ...

PŘÍPRAVA SN-38 z 7-Et-THCPT

[redacted]

Násada: 80 ml 7-Et-THCPT (768-88-1)
22 ml demivody
0,77 g IBDA (š 417379/1)
20 ml Acetonitril

Aparatura: 1hrdlá 250 ml baňka Büchi, RVO, elmag. míchačka, odm. válce, váženka, büchnerka s odsávací zkumavkou.

Postup: Do baňky jsme dali demivodu, přisypali
v 9⁴⁷h IBDA a nakonec zalili 7-Et-THCPT. Barva tmavě žlutá přechází na světle červenohnědou.
768-89-1 v 9⁴⁹ vzorek na HPLC 72,86% SN-38; 15,55% 7-Et-CPT
(60°C) Míchá se 15 minut. Dáno na RVO odpařit. Odpařuje se
RVC = 1h od 10⁰⁰ hod. Dostatečně to probublávalo. Konec v 11¹⁰ hod.
Na odparek nalito 10 ml Acetonitrilu, zhomogenizováno na UZ. Dochlazeno stud. vodou. Filtrace a promytí 2x 10 ml Acetonitrilu.
768-89-2 N = 0,2226 g SN-38 HPLC: 80,22% SN-38; 13,50% 7-Et-CPT
100% výt. => 0,486 g (45,8% výtěžek na 7-Et-CPT)
768-89-3 Matečný - HPLC (3 ml): 39,31% SN-38; 2,82 % 7-Et-CPT

[redacted] Mokulová
Dobrovolný

VÝPOČET MNOŽSTVÍ VODY DO 7-ET-THCPT:
Objem 7-Et-THCPT * 0,282 = x ml demivody

EXHIBIT B

HYDROGENATION OF 7-Et-CPT

[redacted]	Charge: 0.5g 7-Et-CPT (768–65–1) 0.32g 5% Pt/C (batch no. 05002 PI) 0.025 mL DMSO (batch no. 432316/1) 70 mL CH_3COOH (batch no. 20810) Equipment: 0.5L metal autoclave, N_2, H_2, a beaker, a weighing bottle, a graduated cylinder, a micropipette, büchner filter with vacuum pump Procedure: 7-Et-CPT was weighed to a beaker, Pt/C was weighed to a weighing bottle and then it was added to the beaker, it was poured with a part of CH_3COOH and homogenized on US. Then DMSO was added, stirred and transferred to an autoclave. The residues were washed with the remaining CH_3COOH. The autoclave was completed and switched on 500 rpm. The autoclave was washed with N_2 (3x) and H_2 (3x) and then H_2 was switched on. Revolutions were increased to 1000 rpm. Hydrogenation started at 10 am at 50°C, at 10:40 am - 62°C, at 10:52 am – 64.2°C Pressure of H_2 – 5 atm, 1000 rpm, t – 63 - 65°C Thanks to the cooling water, the temperature shows 2°C less, so at 12:40 am the temperature was set to 67°C. At 12:55 am t = 64.9°C The hydrogenation was stopped at 4 pm. Filtered and washed. Now in the fridge.
[redacted] 768–72–1 768–73–1 100% yield → 0.0505g 768–73–2	The suspension solidified in the fridge, it was dissolved in hot water. A sample was taken for HPLC. N about 80 mL 7-Et-THCPT *<page 72 ends, page 73 begins>* Thickened on RVO to an oily consistence. 8 mL of MeOH was added. Crystals were released. Filtration & washing with 10 Ml of MeOH. Drying at 35°C in a drier. N = 0.2385 g 7-Et-CPT. HPLC: 94.50 7-Et-CPT mother liquor HPLC: (20μL): 58.69% 7-Et-THCPT *Illegible signature* [redacted] *Legible signature - Dobrovolný*

HYDROGENATION OF 7-Et-CPT

[redacted]	Charge: 0.5g 7-Et-CPT (768–65–1) 0.32g 5% Pt/C (batch no. 05002 PI) 0.025 mL DMSO (batch no. 432316/1) 70 mL CH_3COOH (batch no. 20810) Equipment: see page 72 Procedure: 7-Et-CPT was weighed to a beaker, Pt/C was weighed to a weighing bottle and then it was added to the beaker, it was poured with a half of the total quantity of CH_3COOH and homogenized on US. DMSO was added. Then it was transferred to an autoclave. The residues were washed with the remaining CH_3COOH. The autoclave was closed and washed with N_2 and H_2. Hydrogenation started at 8:47 am. 900 rpm, pressure of H_2 – 5 atm, heated to 65°C at 9:50 am At 9:10 am = 61.2°C
[redacted]	End of hydrogenation at 9:50 am. The hydrogenation lasted for 24 hours (at 65°C). At 10:45 am filtration and washing with CH_3COOH (5 mL). Something went through, new filtration and washing were necessary (total consumption of about 40 mL of CH_3COOH). 768-84-1 N=124 mL 7-Et-THCPT. Sample HPLC: 64.94% 7-Et-THCPT, 7.975 7-Et-CPT (light orange to dark yellow solution)
	Procedure: At 11:40 am it was poured to a 250 mL 3-neck flask, 35 mL of demiwater was added and IBDA was dosed (stirred with 600 rpm) 1. 0.613g at 11:42 am dark orange after 30 minutes 2. 0.15g at 12:12 am dark orange after 50 minutes 3. 0.13g at 12:55 am dark orange to brown after 30 minutes 4. 0.04g at 1:27 pm dark reddish brown

HYDROGENATION OF 7-Et-CPT

[redacted]	Charge: see page 84 Equipment: see page 72 Procedure: 7-Et-CPT was weighed to a beaker, Pt/C was added to the beaker, it was poured with a part of CH_3COOH and homogenized on US. DMSO was added. Then it was stirred and transferred to an autoclave. The residues were washed with the remaining CH_3COOH. The autoclave was closed, stirring and heating was switched on and it was washed with N_2 and H_2. Hydrogenation started at 1:25 pm. 900 rpm, pressure of H_2 – 5 atm, heated to 65°C at 2:00 pm
[redacted] 768-88-1	End of hydrogenation at 9:30 am. The hydrogenation lasted for 43.5 hours (at 65°C). Cooled to 25°C. Filtration and washing with CH_3COOH N=80 mL 7-Et-THCPT. HPLC (30μL): 65.80% 7-Et-THCPT 8.23% 7-Et-CPT The light yellow solution at the filtration turns to a dark yellow solution. *Illegible signature* [redacted] *Legible signature - Dobrovolný*

PREPARATION OF SN-38 FROM 7-ET-THCPT

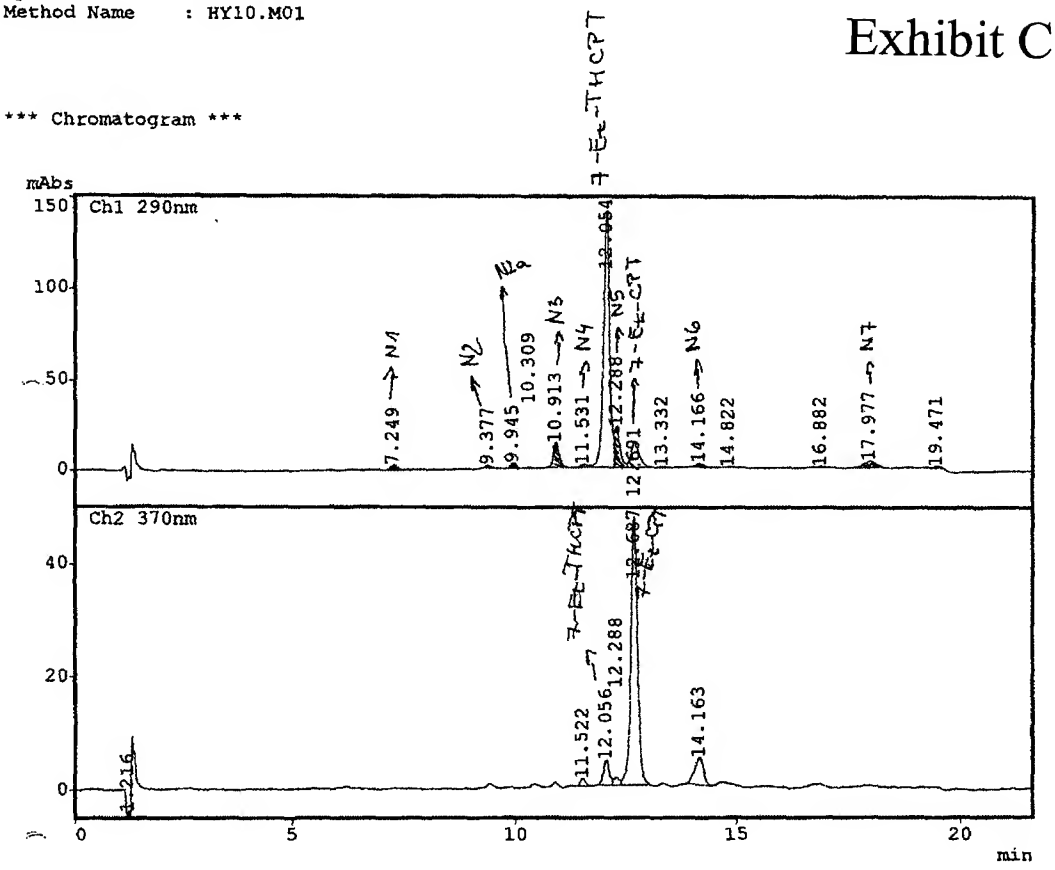
[redacted]	Charge: 80 mL 7-Et-THCPT (768-88-1) 22 mL demiwater 0.77g IBDA (batch no. 417379/1) 20 mL acetonitrile Equipment: a 250-mL 1-neck Büchi beaker, RVO, an elmag. stirrer, a graduated cylinder, a weighing bottle, a büchner filter with vacuum pump Procedure: Demiwater was poured to a flask, IBDA was added and it was poured with 7-Et-THCPT immediately. The dark yellow color turns to light reddish brown.
768-89-1 (60°C) RVO=1Hr	At 9:49 am a sample was taken for HPLC: 72.86% SN-38, 15.55% 7-Et-CPT Stirred for 15 min. Then evaporation on RVO till 10:00 am. End at 11:10 am. 10 mL of acetonitrile poured on the evaporation residue, homogenized on US. Cooled with cold water. Filtration and washing with about 10 mL of acetonitrile.
768-89-2 100%yield→ 0.486g 768-89-3	N=0.2226g SN-38. HPLC: 80.22% SN-38; 13.50% 7-Et-CPT (45.8% yield to 7-Et-CPT) Mother liquor HPLC (30μL): 39.31% SN-38; 25.82% 7-Et-CPT
	Illegible signature [redacted] *Legible signature – Dobrovolný* CALCULATION OF WATER QUANTITY FOR 7-ET-THCPT 7-Et-THCPT volume x 0.282 – x mL demiH_2O

EXHIBIT C

CLASS-LC10 Ver.=1.64A SYS=1 REPORT.NO=8 DATA=HY10.K01 [redacted] 10:04:28
Vial # : 90
Sample : 7-Et-THCPT
ID : 768-88-1
Inj. Volume : 10
Type : Unknown
Detector : SPD-M10A
Operator : Buchta
Method Name : HY10.M01

Exhibit C1

*** Chromatogram ***



*** Peak Report ***

PKNO	ChNO	TIME	AREA	CONC	NAME	HEIGHT	
1	2	1.216	1185			818	
2	1	7.249	26142	1.2689		2752	N1
3	1	9.377	14333	0.6958		1786	N2
4	1	9.945	22365	1.0856		2891	N2a
5	1	10.309	4577	0.2222		544	
6	1	10.913	127527	6.1903		13910	N3
7	1	11.531	20869	1.0130		1486	N4
	2	11.522	10840			1323	
8	1	12.054	1355610	65.8024		140795	7-Et-THCPT
	2	*12.056	40532			4375	
9	1	12.288	196504	9.5385		22707	N5
	2	12.288	13172			1479	
10	1	12.691	169592	8.2321		13461	7-Et-CPT
	2	*12.687	500452			47687	
11	1	13.332	4884	0.2371		474	
12	1	14.166	22919	1.1125		2061	N6
	2	14.163	64983			4773	
13	1	14.822	2725	0.1323		248	
14	1	16.882	10049	0.4878		733	
15	1	17.977	67269	3.2653		3621	N7
16	1	19.471	14759	0.7164		1064	

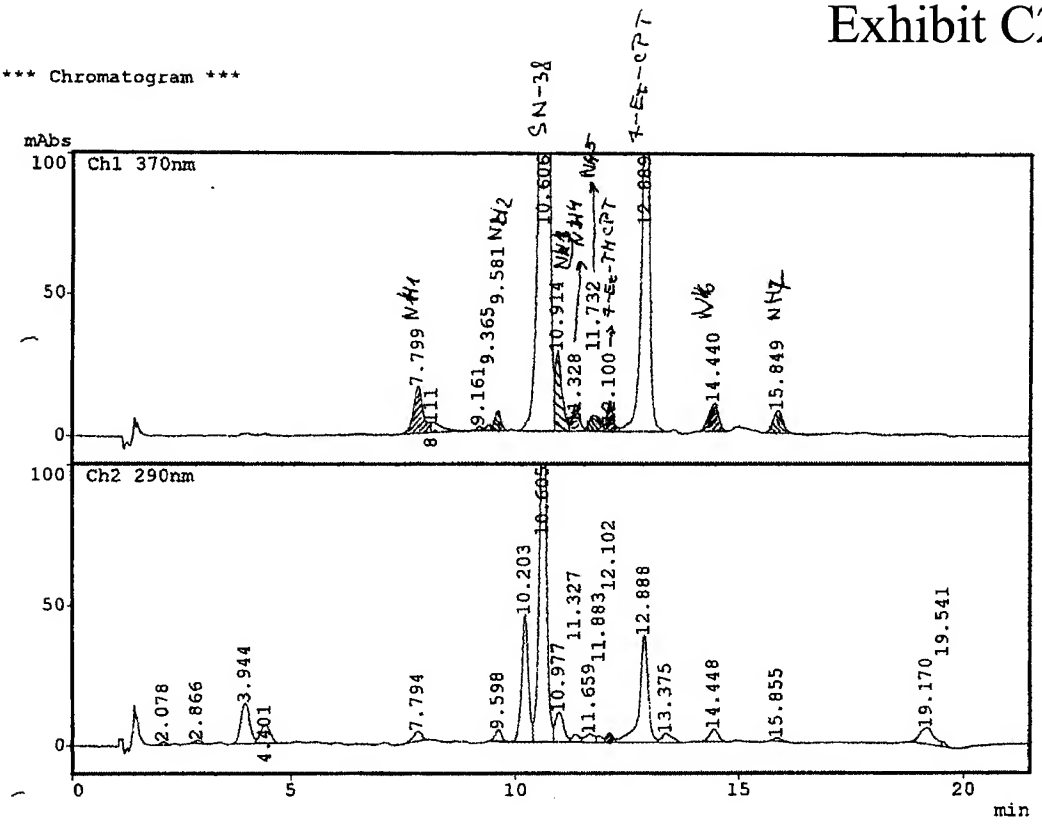
	2691287	100.0000	268988

[redacted]

ID : 768-89-1
Inj. Volume : 25
Type : Unknown
Detector : SPD-M10A
Operator : Buchta
Method Name : HY12.M01

Exhibit C2

*** Chromatogram ***



*** Peak Report ***

PKNO	ChNO	TIME	AREA	CONC	NAME	HEIGHT	
1	2	2.078	9358			1094	
2	2	2.866	12736			1192	
3	2	3.944	203759			14350	
4	2	4.401	100667			6579	
5	1	7.799	237360	2.2196		16259	NH1
	2	7.794	45972			3580	
6	1	8.111	64349	0.6017		3603	
7	1	9.161	11592	0.1084		1492	
8	1	9.365	15867	0.1484		1840	
9	1	9.581	62022	0.5800		6646	NH2
	2	9.598	39517			4055	
10	2	10.203	459287			45696	
11	1	10.606	7791901	72.8629		821960	SN-38
	2	10.605	1704891			182583	
12	1	10.914	295566	2.7639		28025	NH4
13	2	10.977	140194			10367	
14	1	11.328	96857	0.9057		9138	NH4
	2	11.327	29673			2756	
15	2	11.659	41715			2799	
16	1	11.732	96183	0.8994		5265	NH5
17	2	11.883	21211			2187	

<Temporary>

[redacted]

18	1	12.100	101379	0.9480	10547	7-Et-THCPT
	2	12.102	32123		3428	
19	1	12.889	1662846	15.5494	143661	7-Et-CPT
	2	12.888	519494		38394	
20	2	13.375	50151		3144	
21	1	14.440	143330	1.3403	9622	N46
	2	14.448	57551		4477	
22	1	15.849	114677	1.0724	7642	NH2
	2	15.855	13725		1135	
23	2	19.170	125623		5800	
24	2	19.541	11300		1704	
			14312873	100.0000	1401014	

[redacted]

Sample	: SN-38
ID	: 768-89-2
Inj. Volume	: 10
Type	: Unknown
Detector	: SPD-M10A
Operator	: Buchta
Method Name	: HY14.M01

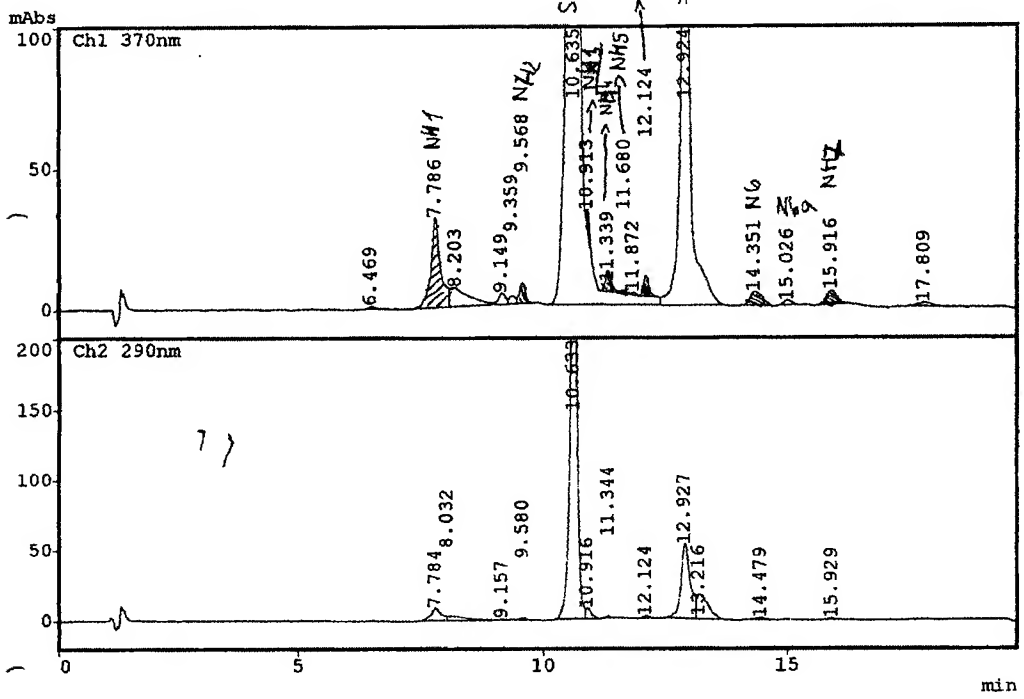
mdzada 0,5g 7-Et-CPT

w 70 ml CH_3COOH

Hydr. 43,5h

Exhibit C3

*** Chromatogram ***



*** Peak Report ***

PKNO	ChNO	TIME	AREA	CONC	NAME	HEIGHT	
1	1	6.469	9579	0.0541		904	
2	1	7.786	432200	2.4426		31618	NH1
	2	7.784	131802			8861	
3	2	8.032	81311			3198	
4	1	8.203	176380	0.9968		6575	
5	1	9.149	43366	0.2451		4058	
	2	9.157	4938			688	
6	1	9.359	26772	0.1513		2978	
7	1	9.568	60383	0.3413		7157	NH2
	2	9.580	10834			1516	
8	1	10.635	14195023	80.2254		1393882	SN-38
	2	10.633	3133537			336897	
9	1	10.913	28174	0.1592		5360	NH4
	2	10.916	58573			7512	
10	1	11.339	60351	0.3411		7228	NH4
	2	11.344	11393			1585	
11	1	11.680	15699	0.0887		1299	NH5
12	1	11.872	6722	0.0380		1038	
13	1	12.124	58092	0.3283		7056	7-Et-THCPT
	2	12.124	16064			2007	
14	1	12.924	2388151	13.4970		186473	7-Et-CPT

[redacted]

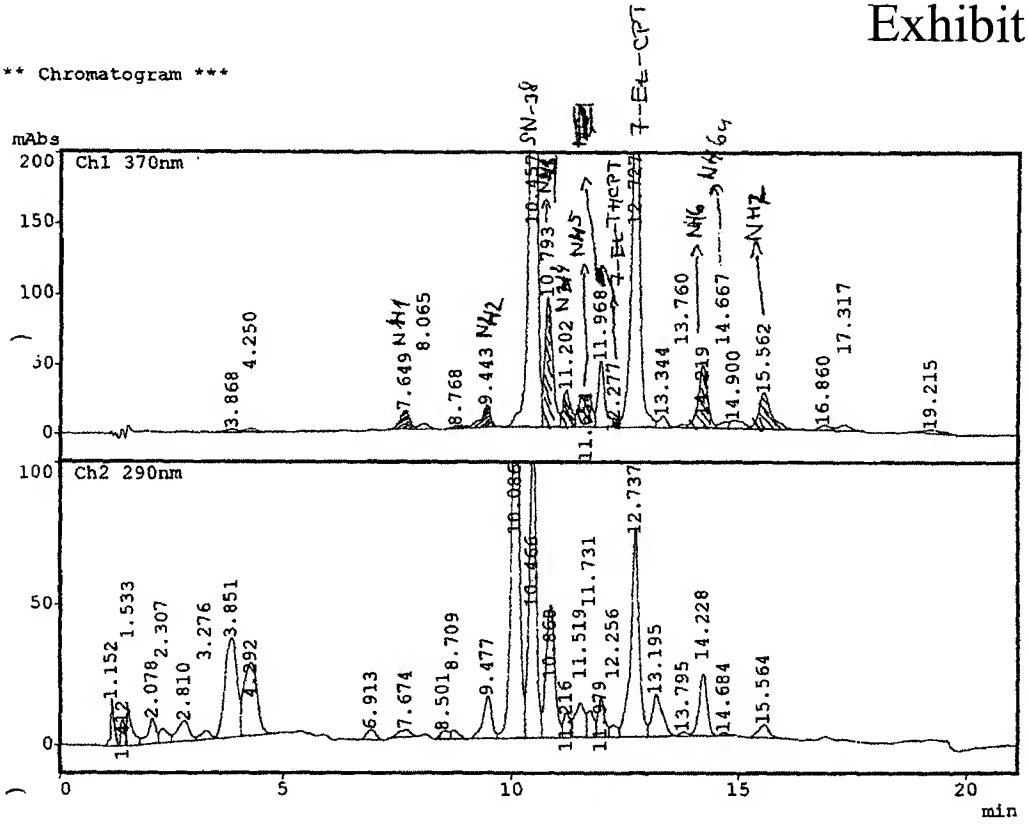
	2	12.927	699268		53199	
15	2	13.216	299481		17251	
16	1	14.351	81994	0.4634	4893	NH6
17	2	14.479	22986		1413	
18	1	15.026	17401	0.0983	1597	NH6a
19	1	15.916	70242	0.3970	4923	NH2
	2	15.929	13901		1104	
20	1	17.809	23403	0.1323	1276	
			22178019	100.0000	2103543	

[redacted]

CLASS-LC10 Ver.=1.64A SYS=1 REPORT.NO=10 DATA=HY15.K01 03/04/04 12:18:09
Vial # : 11
Sample : SN-38-filtrát
ID : 768-89-3
Inj. Volume : 35
Type : Unknown
Detector : SPD-M10A
Operator : Buchta
Method Name : HY15.M01

Exhibit C4

*** Chromatogram ***



*** Peak Report ***

PKNO	ChNO	TIME	AREA	CONC	NAME	HEIGHT	
1	2	1.152	89088			16787	
2	2	1.412	58743			9019	
3	2	1.533	110664			13153	
4	2	2.078	114356			8950	
5	2	2.307	71341			5141	
6	2	2.810	132876			7000	
7	2	3.276	42249			3022	
8	1	3.868	36847	0.2748		2222	
	2	3.851	658300			35348	
9	1	4.250	42287	0.3154		2309	
10	2	4.292	521477			25944	
11	2	6.913	47289			3508	
12	1	7.649	192377	1.4348		13300	NH1
13	2	7.674	47005			2696	
14	1	8.065	60921	0.4544		4274	
15	2	8.501	35963			2624	
16	2	8.709	30255			2885	
17	1	8.768	37335	0.2785		1887	
18	1	9.443	227410	1.6961		15694	NH2
19	2	9.477	210773			15100	
20	2	10.086	1934972			174271	

[redacted]

21	1	10.457	5270514	39.3098	490915	SN-38
	2	10.466	1151863		109897	
22	1	10.793	1101620	8.2164	92234	NH1
23	2	10.868	643056		47453	
24	1	11.202	321031	2.3944	26508	NH4
	2	11.216	96591		8851	
25	2	11.519	180752		12164	
26	1	11.654	462099	3.4465	22140	NH5
27	2	11.731	102865		9400	
28	1	11.968	494503	3.6882	47816	DMCH
	2	11.979	141488		13985	
29	2	12.256	51904		4249	
30	1	12.277	47916	0.3574	6080	7-Et-THCPT
31	1	12.727	3462306	25.8234	283837	7-Et-CPT
	2	12.737	978650		74416	
32	2	13.195	242877		15130	
33	1	13.344	102000	0.7608	7981	
34	1	13.760	27968	0.2086	2282	
35	2	13.795	20045		1626	
36	1	14.219	627090	4.6771	45020	NH6
	2	14.228	273081		21953	
37	1	14.667	61565	0.4592	4472	NH6α
	2	14.684	11515		1224	
38	1	14.900	122053	0.9103	5738	
39	1	15.562	494479	3.6880	25981	NH2
	2	15.564	73277		4520	
40	1	16.860	59809	0.4461	3599	
41	1	17.317	78253	0.5836	4139	
42	1	19.215	77255	0.5762	2634	
			21480951	100.0000	1761376	

[redacted]